

Amendment to the Claims

Claim 1 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising

(a) a nucleic acid sequence having at least 75% 95% sequence identity to the nucleic acid sequence of SEQ ID NO:29,

wherein the nucleic acid encodes a fluorescent polypeptide and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or

(b) a nucleic acid sequence completely complementary to (a).

Claims 2 to 13 (canceled)

Claim 14 (previously presented): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the fluorescent polypeptide comprises a green fluorescent protein.

Claim 15 (previously presented): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the fluorescent polypeptide comprises a cyan fluorescent protein.

Claims 16 to 28 (canceled)

Claim 29 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the nucleic acid comprises a sequence that hybridizes under high stringency conditions to a sequence comprising

(a) the nucleic acid sequence of SEQ ID NO:29,

wherein the isolated, synthetic or recombinant nucleic acid encodes a fluorescent polypeptide, and the ~~stringent~~ high stringency conditions include a wash step comprising a wash in 0.1X SSC, 0.5% SDS for 15 to 30 minutes at 68°C

and the nucleic acid sequence has at least 90% 95% sequence identity to the nucleic acid sequence of SEQ ID NO:29; or

(b) a nucleic acid sequence completely complementary to (a).

Claims 30 to 32 (canceled)

Claim 33 (currently amended): A nucleic acid probe for identifying or isolating a nucleic acid encoding a fluorescent polypeptide, wherein the probe comprises at least 10 consecutive bases of a sequence comprising:

(a) the nucleic acid sequence of SEQ ID NO:29,

where in the probe identifies the nucleic acid by binding or hybridization under stringent conditions, and the stringent conditions include a wash step comprising a wash in 0.2x SSC at a temperature of about 65°C for about 15 minutes

and the nucleic acid sequence has at least ~~75%~~ 95% sequence identity to the nucleic acid sequence of SEQ ID NO:29; or

(b) a nucleic acid sequence completely complementary to (a).

Claim 34 (canceled)

Claim 35 (previously presented): A nucleic acid probe for identifying a nucleic acid encoding a fluorescent polypeptide, wherein the probe comprises the nucleic acid sequence of claim 1.

Claims 36 to 39 (canceled)

Claim 40 (previously presented): An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide with a fluorescent activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence of claim 1.

Claim 41 (canceled)

Claim 42 (withdrawn): A method of amplifying a nucleic acid encoding a fluorescent polypeptide comprising amplification of a template nucleic acid with an

amplification primer sequence pair capable of amplifying a nucleic acid sequence comprising a sequence of claim 1.

Claim 43 (previously presented): An expression cassette comprising a nucleic acid of claim 1.

Claim 44 (previously presented): A vector comprising a nucleic acid of claim 1.

Claim 45 (previously presented): A cloning vehicle comprising the vector of claim 44, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

Claims 46 to 47 (canceled)

Claim 48 (previously presented): A transformed cell comprising a vector, wherein the vector comprises a nucleic acid of claim 1.

Claim 49 (previously presented): A transformed cell comprising a nucleic acid of claim 1.

Claim 50 (canceled)

Claim 51 (withdrawn): A transgenic non-human animal comprising the nucleic acid of claim 1.

Claims 52 to 53 (canceled)

Claim 54 (withdrawn): A transgenic plant comprising the nucleic acid of claim 1.

Claim 55 (canceled)

Claim 56 (withdrawn): A transgenic seed comprising the nucleic acid of claim 1.

Claim 57 (canceled)

Claim 58 (withdrawn): An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to the nucleic acid of claim 1.

Claims 59 to 86 (canceled)

Claim 87 (previously presented): An array comprising the immobilized nucleic acid of claim 1.

Claims 88 to 105 (canceled)

Claim 106 (withdrawn): A method for identifying a feature in a sequence comprising the steps of:

(a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a nucleic acid sequence, wherein the nucleic acid sequence comprises the sequence of claim 1, and

(b) identifying one or more features in the sequence with the computer program.

Claim 107 (withdrawn): A method for comparing a first sequence to a second sequence comprising the steps of:

(a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises the sequence of claim 1; and

(b) determining differences between the first sequence and the second sequence with the computer program.

Claims 108 to 110 (canceled)

Claim 111 (withdrawn): A method for isolating or recovering a nucleic acid encoding a polypeptide with a fluorescent activity from an environmental sample comprising the steps of:

(a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide with a fluorescent activity, wherein the primer pair is capable of amplifying the nucleic acid of claim 1;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,

(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a fluorescent polypeptide from an environmental sample.

Claim 112 (canceled)

Claim 113 (withdrawn): A method for isolating or recovering a nucleic acid encoding a polypeptide with a fluorescent activity from an environmental sample comprising the steps of:

(a) providing a polynucleotide probe of claim 33 or claim 35;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a fluorescent activity from an environmental sample.

Claims 114 to 115 (canceled)

Claim 116 (withdrawn): A method of generating a variant of a nucleic acid encoding a fluorescent protein comprising the steps of:

- (a) providing a template nucleic acid comprising the nucleic acid of claim 1; and
- (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

Claims 117 to 137 (canceled)

Claim 138 (withdrawn): A method for modifying codons in a nucleic acid encoding a fluorescent polypeptide to increase its expression in a host cell, the method comprising

- (a) providing a nucleic acid encoding a fluorescent polypeptide of claim 1; and
- (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

Claims 139 to 142 (canceled)

Claim 143 (withdrawn): A method for producing a library of nucleic acids encoding a plurality of modified fluorescent polypeptide active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising:

(a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence of claim 1, and the nucleic acid encodes a fluorescent polypeptide active site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified fluorescent polypeptide active sites.

Claims 144 to 173 (canceled)

Claim 174 (withdrawn): A method for using a nucleic acid encoding a fluorescent polypeptide in gene therapy comprising the following steps:

(a) obtaining from a patient a viable sample of primary cells of a particular cell type;

(b) inserting in the cells of step (a) a nucleic acid segment encoding a desired gene product;

(c) introducing in the cell of step (b) a vector comprising a nucleic acid of claim 1;

(d) identifying and isolating cells or cell lines that express the gene product of step (b);

(e) re-introducing the cells that express the gene product;

(f) removing from the patient an aliquot of tissue including cells resulting from step (d) and their progeny;

(g) determining the quantity of the cells resulting from the step (d) in the aliquot of step (f), thereby the introduction of the vector of step (c) in addition to the desired gene allows the identification of viable cells that contain and express the desired gene of step b.

Claim 175 (withdrawn): A method of gene therapy comprising the following steps:

- (a) providing a plurality of tissue cells;
- (b) providing a retroviral vector encoding a desired gene product;
- (c) providing a vector comprising the nucleic acid of claim 1; and
- (d) contacting the target cells of step (a) with the retroviral vectors of step (b) and a vector of step (c) under conditions wherein the cells of step (a) are transfected with the vectors of steps (b) and (c) allowing co-expression of the nucleic acid, thereby allowing assessment of proportion of transfected cells and levels of expression.

Claim 176 (canceled)

Claim 177 (withdrawn): A method for diagnostic testing comprising the following steps:

- (a) providing a nucleic acid of claim 1;
- (b) placing the nucleic acid of step (a) under control of a promoter;
- (c) providing an inducing agent to induce the promoter of step (b); and
- (d) contacting the agent of step (c) with the promoter of step (b) under condition wherein the agent of step (c) induces the promoter of step (b), thereby causing the expression of a fluorescent polypeptide in cells, cell lines or tissues, wherein the cells, cell lines or tissue will become fluorescent in the presence of the inducing agent.

Claims 178 to 181 (canceled)

Claims 182 (withdrawn): A method for assessing the effect of selected culture components and conditions on selected gene expression comprising the following steps:

- (a) providing a cell comprising a nucleic acid of claim 1 operably linked to a regulatory sequence derived from a selected gene;
- (b) incubating the cell of step (a) under selected culture conditions or in the presence of selected components, thereby expressing a polypeptide encoded by the nucleic acid of step (a); and

(c) detecting the presence and subcellular localization of fluorescent signal thereby assessing the effect of selected cultures components or condition on selected gene expression.

Claim 183 (canceled)

Claim 184 (withdrawn): A method for assessing a mutagenic potential of a test agent in a tissue culture or transgenic animal comprising the following steps:

(a) providing the nucleic acid of claim 1 operably linked to a transcriptional control element, wherein the transcription control element can be negatively regulated by a repressor;

(b) providing a repressor under control of a constitutively expressed gene;

(c) providing a test compound capable of interacting with a promoter of the constitutively expressed gene thereby turning it off; and

(d) contacting the test agent of step (c) with the repressor of step (b) under conditions wherein the test agent inactivates or turns off the gene expressing the repressor thereby causing the expression of the nucleic acid of step (a).

Claims 185 to 186 (canceled)

Claim 187 (withdrawn): A method for identifying a compound capable of changing expression of a target gene comprising of the following steps:

(a) providing a first nucleic acid having a sequence of 1 or claim 29 and expressing a first polypeptide, wherein the nucleic acid is operably linked to a promoter of a target gene in a cell;

(b) providing a second nucleic acid having the sequence of claim 1, and expressing a second polypeptide, wherein the second nucleic acid is operably linked to a promoter of a constitutively expressed gene in a cell, wherein the first polypeptide emits a light at a wavelength different than the wavelength of the light emitted by the second polypeptide;

(c) providing a compound affecting the expression of the target gene of step (a) by binding to the promoter of the target gene;

(d) contacting the compound of step (c) with the cell of step (a);

(e) expressing the first and second polypeptide, and

(f) detecting fluorescence of the first and second polypeptides,

(i) wherein altered fluorescence of the first polypeptide and unchanged fluorescence of the second polypeptide demonstrates that the compound binds to the target gene promoter and has no non-specific or cytotoxic effects thereby not altering expression of the second polypeptide; or

(ii) wherein altered fluorescence of the first polypeptide and altered fluorescence of the second polypeptide demonstrates that the test drug has non-specific or cytotoxic effects thereby affecting the expression of the second polypeptide.

Claim 188 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising a sequence encoding a fluorescent protein and having at least ~~75%~~ 95% sequence identity to the sequence of SEQ ID NO:29.

Claim 189 (previously presented): An isolated, synthetic or recombinant nucleic acid comprising a sequence as set forth in SEQ ID NO:29.

Claim 190 (withdrawn): An isolated, synthetic or recombinant nucleic acid encoding a polypeptide comprising the sequence of SEQ ID NO:30.

Claim 191 to 202 (canceled)

Claim 203 to 206 (canceled): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence has at least 80% sequence identity to the sequence of SEQ ID NO:29.

Claim 207 (previously presented): The isolated, synthetic or recombinant nucleic acid of claim 206, wherein the nucleic acid sequence has at least 98% sequence identity to the sequence of SEQ ID NO:29.

Claim 208 (withdrawn): The isolated, synthetic or recombinant nucleic acid of claim 207, wherein the nucleic acid sequence is the sequence of SEQ ID NO:17.

Claims 209 to 214 (canceled)

Claim 215 (withdrawn): The isolated, synthetic or recombinant nucleic acid of claim 203, wherein the nucleic acid sequence is the sequence of SEQ ID NO:187, SEQ ID NO:65, SEQ ID NO:93, SEQ ID NO:189, SEQ ID NO:61, SEQ ID NO:53, SEQ ID NO:145, SEQ ID NO:45, SEQ ID NO:173, SEQ ID NO:177, SEQ ID NO:155, SEQ ID NO:183, SEQ ID NO:33, SEQ ID NO:129, SEQ ID NO:175 or SEQ ID NO:161.

Claim 216 (withdrawn): The isolated, synthetic or recombinant nucleic acid of claim 204, wherein the nucleic acid sequence is the sequence of SEQ ID NO:157 or SEQ ID NO:149.

Claim 217 (previously presented): A recombinant nucleic acid encoding a fluorescent protein made by a process comprising the following steps:

(a) providing a template nucleic acid comprising at least the sequence of claim 1;
and

(b) generating a recombinant nucleic acid encoding a fluorescent protein by subjecting the template nucleic acid to Synthetic Ligation Reassembly (SLR).

Claim 218 (currently amended): The recombinant nucleic acid of claim 217, further comprising a step [(c)] (c) expressing the assembled fluorescent protein-encoding nucleic acid of [(d)] (b) to generate a fluorescent protein.

Claim 219 (withdrawn): The recombinant nucleic acid of claim 217, wherein the template nucleic acid comprises the nucleic acid sequence of SEQ ID NO:17.

Claim 220 (withdrawn): The recombinant nucleic acid of claim 217, further comprising at least a second template nucleic acid comprising the nucleic acid sequence of SEQ ID NO:101, SEQ ID NO:125, SEQ ID NO:87, SEQ ID NO:103, SEQ ID NO:135, SEQ ID NO:119, SEQ ID NO:131, SEQ ID NO:167, SEQ ID NO:73, SEQ ID NO:137, SEQ ID NO:127, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:89, SEQ ID NO:153, SEQ ID NO:69, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:59, SEQ ID NO:77, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:185, SEQ ID NO:41, SEQ ID NO:143, SEQ ID NO:51, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:43, SEQ ID NO:99, SEQ ID NO:83, SEQ ID NO:95, SEQ ID NO:113, SEQ ID NO:123 or SEQ ID NO:165.

Claim 221 (withdrawn): A method for making a nucleic acid encoding a fluorescent protein comprising the following steps:

- (a) providing a template nucleic acid comprising at least the sequence of claim 1;
and
- (b) generating a recombinant nucleic acid encoding a fluorescent protein by subjecting the template nucleic acid to Synthetic Ligation Reassembly (SLR).

Claim 222 (withdrawn): The method of claim 221, further comprising the step (c) expressing the assembled fluorescent protein-encoding nucleic acid of (d) to generate a fluorescent protein.

Claim 223 (withdrawn): The method of claim 221, wherein the template nucleic acid comprises the nucleic acid sequence of SEQ ID NO:17.

Claim 224 (withdrawn): The method of claim 221, further comprising at least a second template nucleic acid comprising the nucleic acid sequence of SEQ ID NO:101, SEQ ID NO:125, SEQ ID NO:87, SEQ ID NO:103, SEQ ID NO:135, SEQ ID NO:119,

SEQ ID NO:131, SEQ ID NO:167, SEQ ID NO:73, SEQ ID NO:137, SEQ ID NO:127, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:89, SEQ ID NO:153, SEQ ID NO:69, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:59, SEQ ID NO:77, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:185, SEQ ID NO:41, SEQ ID NO:143, SEQ ID NO:51, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:43, SEQ ID NO:99, SEQ ID NO:83, SEQ ID NO:95, SEQ ID NO:113, SEQ ID NO:123 or SEQ ID NO:165.

Claim 225 (currently amended): A recombinant nucleic acid encoding a fluorescent protein codon-optimized for expression in a host cell, made by the process of ~~claim 138~~

(a) providing a nucleic acid encoding a fluorescent polypeptide of claim 1; and
(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

Claim 226 (previously presented): An isolated, synthetic or recombinant nucleic acid encoding a fluorescent protein comprising the sequence of claim 1, and further comprising codons optimized for expression in a host cell.

Claim 227 (previously presented): An isolated, synthetic or recombinant nucleic acid encoding a fluorescent protein comprising the sequence of claim 1, and further comprising coding sequence for a tag or a reporter sequence, and optionally the tag or reporter sequence comprises an epitope tag, a fluorescent tag, a genetic reporter or a protein tag.

Claim 228 (previously presented): The nucleic acid probe of claim 35, further comprising an epitope tag or a fluorescent tag.

Claim 229 (withdrawn): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence is the sequence of SEQ ID NO:101, SEQ ID NO:125, SEQ ID NO:87, SEQ ID NO:103, SEQ ID NO:135, SEQ ID NO:119, SEQ ID NO:131, SEQ ID NO:167, SEQ ID NO:73, SEQ ID NO:137, SEQ ID NO:127, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:89, SEQ ID NO:153, SEQ ID NO:69, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:59, SEQ ID NO:77, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:185, SEQ ID NO:41, SEQ ID NO:143, SEQ ID NO:51, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:43, SEQ ID NO:99, SEQ ID NO:83, SEQ ID NO:95, SEQ ID NO:113, SEQ ID NO:123 or SEQ ID NO:165.

Claim 230 (withdrawn): The isolated, synthetic or recombinant nucleic acid of claim 206, wherein the nucleic acid sequence is the sequence of SEQ ID NO:17.

Claim 231 (withdrawn): An isolated, synthetic or recombinant nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:30.